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COMPLETE SPECIFICATION.

Therapeutic Compositions Containing β-Alanine.

We, A.E.C. SOCIETE DE CHIMIE ORGANIQUE ET BIOLOGIQUE, a French Body Corporate of Commentry (Allier) France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to therapeutic compositions containing β-alanine.

β-Alanine is a compound chemically defined by the empirical formula: $C_3H_7NO^2$ (molecular weight 89.09) and the structural formula:—

$$H_2N-CH_2-CH_2-COOH$$

Its percentage composition corresponds to:—

C = 40.44%	H = 7.92%
N = 15.72%	O = 35.92%

It has the following physical properties: it is a white powder, soluble in water whence it crystallizes in orthorhombic pyramids, slightly soluble in alcohol, practically insoluble in ether and acetone.

The dissociation constants of β-alanine are:

$pK^1_1(COOH)$: 3.60, pK^1_2 (NH_3^+) : 10.19,

the isoelectric *p*H being 6.90.

β-Alanine has an indefinite melting point since it decomposes between 195 and 205° C.; it is sublimated moreover from 111° C. onwards under 1.3 mm. of mercury.

β-Alanine can be identified by the following reactions carried out on a solution of 0.50 g. of the compound in 5 ml of distilled water.

(a) Add to 10 g. of this aqueous solution 11 drops of 1% ninhydrin aqueous solution. Place on a boiling water bath. A fine violet colour is very rapidly produced.

(b) Add to 2 ml. of this solution 2 ml. of the aqueous solution of phosphotungstic acid (R). A white cloud is produced. Then add 5 ml. of 20% hydrochloric acid.

After 20 to 30 seconds, a white precipitate progressively appears which rapidly collects at the bottom of the tube.

The purity of β-alanine can be verified by the following titration methods:—

(a) Total nitrogen titration by the method indicated in the French Codex, 1949, page 1968.

(b) Amino nitrogen titration by the potentiometric method. This method has been described by VIGNERON (Annales Pharmaceutiques françaises 1960, *18*, 404).

A description of a process of preparing β-alanine will now be given by way of example.

Pour into a 2 litre flask provided with a stirrer and a thermometer and a dropping funnel which permits drop by drop affusions and is placed under a hood, 185 g. of barium hydroxide ($Ba(OH)_2$, 8 H_2O).

Heat on a water bath.

When the barium hydroxide is dissolved in its water of crystallization, start up the stirrer and add drop by drop over a period of 40 minutes 70.1 g. of β-amino-propionitrile.

Thereafter maintain the temperature at 90 to 95° C. for 40 minutes.

Add 40 g. of asbestos and a litre of hot water.

Saturate with carbon dioxide while maintaining the temperature at 85 to 90° C.

Filter on a Buchner funnel. Drain.

Put the drained cake back into the flask with 500 ml. of hot water.

Heat the mixture for 20 minutes while stirring.

Filter. Once more wash with 500 ml. of

hot water. Concentrate the filtrate and the washing waters on a water bath under low pressure until precipitation.

Recover the residue with 200 ml. of hot water and 0.50 g. of discolouring carbon black. Heat on a water bath for a few minutes.

Filter in an Erlenmayer flask weighted with 500 ml. and heat on a water bath while injecting compressed air on the surface of the solution.

When the solution is reduced to 130 g., cool to 15—20° C. and slowly dilute with 400 ml. of methanol. After a few hours in the refrigerator drain and wash twice with 100 ml. of methanol.

The yield is 75 g.

The pharmacological study of β-alanine gives the following results:—

A—*Toxicity.*

The toxicity of β-alanine was studied by the oral and intravenous routes in the Swiss mouse having a mean weight of 20 g. The product was administered in aqueous solution.

The LD_{50} are the following:—

By the intravenous route: about 5 g./kg.
By the oral route: exceeding 10 g./kg.

B—*Physiological Behaviour.*

This product was studied in respect of the actions it has on the various phenomena of anaphylaxis, redness, temperature rise, respiratory disorders, paresthesia. These various phenomena can apparently be assembled under a common histaminic process set off in particular either by allergy or by the absorption of nicotinic derivatives.

The conditions in which various reactions occur in man can be reproduced principally in the guinea-pig, the rabbit and the dog which are much more sensitive than the rat, the mouse, etc.

Three examples will be given:—

(a) Intolerances were produced in the guinea-pig at high dose by the administration of nicotinic derivatives. The temperature rise of the ear was measured, it manifesting in an intense peripheral vasodilatation. Prior oral administration of β-alanine inhibits these reactions.

(b) Guinea-pigs having previously received β-alanine by the oral route were injected with histamine by the subcutaneous rate and the intraperitoneal route. By comparison with reference animals, it was observed that the previously administered β-alanine has under these conditions a certain protective action of the anti-histaminic type.

(c) Whereas in the rabbit anaesthetized with urethane, the intra venous injection of histamine produces a definite hypotension, the oral administration of β-alanine materially decreases the amplitude of the hypotension.

β-Alanine also acts on the contractions of the ileum of the guinea-pig when this guinea-pig has been previously rendered sensitive by an allergen.

These tests reveal that the activity of β-alanine is that of an indirect anti-histaminic and anti-allergic acting principally on the peripheral receptors affording evidence of phenomena producing allergic reactions. It can also act on the production of endogenous histamine.

Whence follow the therapeutic indications of β-alanine which can be described for treating allergic and histaminic processes on the whole as well as flushes which accompany the discharges of seratine in the carcinoid tumours.

In this respect clinical tests revealed the following actions of β-alanine:—

1. Action against sudden flushes.

Several tests consisting in the administration of β-alanine to women having passed the menopause and subject to many sudden flushes resulted in a considerable and spectacular reduction of these flushes. It should be mentioned in this respect that β-alanine has no structural relation to estrogens, androgens or in a general way to hormonal derivatives and that it can be administered by the oral route.

2. Protection against redness and congestive phenomena caused by nicotinic acid, and its salified or esterified derivatives.

It has been observed that from the experimental point of view and clinical point of view the administration of β-alanine prevents congestive phenomena of redness or paresthesia which sometimes interferes with the administration of nicotinic derivatives.

3. Action against reactions of the allergic type.

Recent experiments revealed in particular that in the case of a penicillic allergy β-alanine stopped in 30 minutes an alarming respiratory and vasomotor cutaneous process. An infinitely small dose of penicillin administered intradermally having produced further incidents, the administration of β-alanine once more stopped these incidents in a very short period of time of around 15 minutes.

It seems that the histaminic discharge produced by intolerance to penicillin appeared but was unable to act on the cutaneous, respiratory, vasomotor, or sympathic or even the suprarenal receptors, as usually occurs.

To summarize, β-alanine provides a very effective protection in man against vasomotor or general cutaneous incidents which

are usually attributed to an excess of histaminemia:—

— Whether these accidents are grouped under the heading of sudden flushes related to the menopause;

— Or produced in certain cases by the administration of nicotinic acid and pharmaceutically acceptable salified or esterified derivatives thereof and in particular lysine nicotinate, with which the β-alanine can thus be admixed so as to avoid their secondary effects, as will be seen hereinafter;

— Or classed under the heading of the classical allergic disorder, this itself having an alimentary, respiratory, cutaneous or therapeutic origin.

As β-alanine has a greater action than estrogens, on the one hand, and classical anti-histaminic products, on the other, it can be considered as exerting an action of protection of the receptors against histaminic discharges, irrespective of their origin.

In these various indications, β-alanine is advantageously administered by the oral route although the other administration routes are not excluded. (In these latter cases the vehicle when liquid is a sterile injectable liquid or contains a flavouring agent.) For administration by the oral route, the active principle can be put into the form of tablets, or other forms suitable for this administration route, with the usual excipients.

An appropriate dose corresponds to the daily administration of 0.02—0.50 g. of active principle. This however is a mean dose which can be exceeded without inconvenience since β-alanine is practically non-toxic.

The oral administration of β-alanine renders it of particular interest in that it permits avoiding anti-histaminic injections or, in other cases, hormonal injections.

It might be mentioned that after surgical castration in the woman due to a malignant disorder, the administration of estrogens being strictly contra-indicated and the administration of male hormones often being insufficient or accompanied by very disagreeable side effects, β-alanine constitutes a preferred treatment.

As has been indicated hereinbefore, a β-alanine-lysine nicotinate admixture is particularly advantageous since it combines the properties of lysine nicotinate and those of β-alanine, the latter furthermore eliminating the secondary effects of said nicotinate.

The LD_{50} of said admixture is, by the oral route: 19.35 g./kg.

The admixture is therefore not at all toxic for the animal.

In man, as opposed to the animal, lysine nicotinate can produce in respect of certain temperaments slight personal intolerences: redness, flushing evoking a histaminic reaction type.

The addition of β-alanine attenuates and decreases the frequency of these intolerances.

The pharmaceutical study of such an admixture reveals the following properties:—

Action on the cholesterolemia produced in the rabbit by the oral administration of high doses of cholesterol.

In the over load period, it limits the hypercholesterolemic rise.

After the period of overload, corresponding in the clinic to the treatment of hypercholesterolemia, it results in a rapid drop in the cholesterol level in the blood serum.

Action on the intense peripheral vasodilatation produced by high doses of nicotinic acid (measure of the temperature of the ear in the guinea-pig).

Action on the endogenous histamine liberated by the histamino-liberators (indirect anti-histaminic acting principally on the peripheral receptors affording evidence of phenomena producing allergic reactions).

The physiological functions are not disturbed following on the administration of the product.

The clinical study of the β-alanine-lysine nicotinate admixture showed that it has a definite action on the manifestations of the atheroma (arteritis, visual disorders, neurological accidents, etc.) which is revealed by biological modifications indicated by the cholesterol level and by the tests of Burnstein and Kunkel.

Action on the manifestations of the atheroma.

— *Arteritis.*

Improvements in the arterial oscillations coupled with clinical improvements were observed, and similar results in respect of cramp or claudication of the lower members in patients who were not immobilized.

— *Visual disorders.*

Several observations revealed functional improvements of the sight measurable by observed less tiredness, improved reading or easier seeing.

— *Action on neurological accidents.*

Observations show numerous remarkably rapid improvements of paralysis and of language in respect of cerebral softening.

A case of epilepsy of vascular origin showed a rapid and surprising improvement in the electroencephalogram.

— *Improvement in general phenomena.*

Further, there was an improvement in renal phenomena in diabetic cases with a drop in the albumin content and drops in

lipoproteins in young atheromatous subjects who did not respond to an active and prolonged thyrotherapy.

— *Modification of biological tests.*

β-Alanine-lysine nicotinate produced the following biological effects:—

High cholesterol levels (exceeding 3 g.) were definitely lowered.

More moderate cholesterol levels were influenced to a lesser extent.

No level was obtained which was below the normal level.

The lipoprotein levels (determined by Burstein's tests) are regularly and substantially influenced (above all the β-lipoproteins).

In conclusion, the clinical results of the admixture are characterized both by the clinical activity of lysinenicotinate and by less vasomotor intolerance incidents due to the effect of β-alanine.

The β-alanine-lysine nicotinate admixture can comprise the two constituents in any relative proportions, these being determined as a function of the proposed therapeutic applications.

By way of example, to which the invention is not limited, the following admixture can be mentioned:—

Lysine nicotinate	...	0.20 g.
β-alanine	...	0.10 g.

The preferred administration form of the admixture is by the oral route, but the other forms of administration are not excluded. For the administration the constituents are mixed with a therapeutically administrable vehicle.

A particularly appropriate pharmaceutical form (to which the invention is not limited) is a double-layer tablet whose structure is the following:—

(a) A central core of lysine nicotinate protected or guaranteed by several layers of gluten varnish which only disintegrates inside the intestine.

Formula of the central core:—

Lysine nicotinate	...	0.20 g.
Excipient	...	0.02 g.
Gluten varnish	...	0.07 g.

(b) A surface layer of β-alanine which is liberated as soon as it is absorbed in the digestive tract.

Formula of the surface layer:—

β-alanine	...	0.10 g.
Excipient	...	0.60 g.

Weight of the finished tablet: 1 g. approx.

The special arrangement just mentioned is to insure a time delay of about two hours between the liberation of the β-alanine and that of the lysine nicotinate which is consequently protected by a prior action of the β-alanine.

The usual dosage of the admixture varies between 6 tablets (starting or attack dose) and 3 tablets (maintenance dose), but this dosage can be modified in accordance with the cases and the therapeutic applications.

WHAT WE CLAIM IS:—

1. Therapeutic composition comprising β-alanine having the formula:—

$$H_2N-CH_2-CH_2-COOH$$

and a therapeutically administrable vehicle which is either a solid, a liquid containing a flavouring agent, or a sterile injectable liquid.

2. Composition as claimed in Claim 1, put in the form of unit doses.

3. Composition as claimed in Claim 2, in the form of tablets.

4. Composition as claimed in Claim 1, further comprising nicotinic acid, or a pharmaceutically acceptable salt or ester thereof.

5. Composition as claimed in Claim 4, in which the nicotinic compound is lysine nicotinate.

6. Composition as claimed in Claim 5, put in the form of tablets comprising a central core containing the lysine nicotinate surrounded by layers of gluten varnish which disintegrates only in the intestine, and a surface layer containing the β-alanine, which is liberated immediately after absorption of the tablet in the digestive tract.

7. Composition as claimed in Claim 6, wherein each tablet contains 0.10 g. of β-alanine and 0.20 g. of lysine nicotinate.

8. Composition according to Claim 1., substantially as described.

MARKS & CLERK,
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